



# ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLE BY SONOCHEMICAL METHOD AND GREEN METHOD USING ZINGIBER OFFICINALE

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## Abstract

Nanoparticles have made a steady progress in all the branches of science. It is used in biological applications including nanomedicine. Zinc oxide is also known as Zincite generally seen in a crystalline form. Zinc oxide nanoparticles are multifunctional. It has effective antibacterial activity. This study focuses on the synthesis of zinc oxide nanoparticle by the sonochemical and green method, characterized by XRD, SEM and to determine the antibacterial efficacy of green and chemical techniques. Results prove that green synthesized Zinc oxide nanoparticle shows the enhanced biocidal activity. In addition the current study has demonstrated that the particle size variation and surface area to volume ratio of green synthesized Zinc oxide nanoparticles are responsible for significant high antibacterial activity. From the result obtained it suggested that the biogenic green fabrication is a better choice due to eco-friendliness.

**Key words:** Antibacterial, efficacy, green fabrication, sonochemical, zincite.

## INTRODUCTION

Nanotechnology is a 'wonder of modern medicine'. It deals with the materials of nano scaled size [1,2] that exhibit novel, chemical, biological properties and functionality. Before the discovery of nanoparticle the doctors preferred antibiotics to destroy the disease causing organisms. But today, nanoparticles have worked wonders. It has got the capacity to kill 650 cells [3]. Metal nanoparticles are observed in bulk size [4,5] extensive catalytic, optical, electronic, magnetic, anti-microbial and anti-inflammatory properties.[6,7]

Zinc oxide nanoparticle is an inorganic nano crystalline metal oxide that can be prepared with extremely high surface areas and are apt for biological applications. The inorganic antibacterial materials shows less toxicity and greater selectivity and heat resistance.[8] chemical methods of synthesis are expensive and require hard labour and time. Large quantities of secondary waste are generated resulting from the addition of chemical agents for precipitate and reduction in the process.[9] They also require the use of chemical compounds, organic solvents as reducing agents.[10,11]

Green synthesis of nanoparticles aims to protect the environment and maintain cleanliness by inventing new chemical process. It depends on plant source and organic compound in the crude extract. It has no destructive particles to help in building better product.<sup>[12]</sup> Green chemistry helps to reduce pollution. [13,14] Green synthesis techniques make use of pollutant free chemicals.

The present study reports that Zinc oxide nanoparticles can be used effectively for the control of microorganism and prevention of infections caused by Staphylococcus aureus and E.Coli. Staphylococcus aureus a gram positive microorganism is a causative agent of nosomial infections. It is accompanied by large amount of pus. [15] E.coli leads to inflammation of the colon and gives to diarrhoea and abdominal pain with bloody stools. It survives in acidic food as its infective dose is as low as 10-100 cells. Leafy vegetables such as spinach and lettuce, pea salad, raw milk, roast beef are suggested by the doctors for the illness caused by E.Coli.

The rhizome of Zingiber officinale is commonly called Ginger belongs to family Zingiberaceae. It is one of the important medicinal plants which is cultivated in countries like India, China, South east Asia and other parts of world.[16] It possess antibacterial, anti cancer, anti-oxidant and anti-diabetic, nausea and cold. The rhizome is a useful part of the plant. It produces an orchid like flower that are greenish yellow streaked with purple colour.[17]

## MATERIALS AND METHODS

Zinc acetate dihydrate and sodium hydroxide was used as the introductory material supplied by Sigma Aldrich chemicals. Zingiber officinale rhizome bought from local market shade dried and powdered. The powder submitted to successive extraction by Soxhlet apparatus sequentially with hexane, pet ether, ethyl acetate, ethanol and water separately in order to extract polar and non polar compounds. Zinc oxide nanoparticle was prepared by two different methods.

**Green synthesis method:** To 50 ml of ethanolic ginger extract 0.02 M zinc acetate dihydrate was added under constant stirring. After 10 minutes stirring 2M NaOH was added to make pH 12 resulted in a white precipitate solution. This was then placed in a magnetic stirrer for 2 hrs. The white precipitate was taken out and washed over and over again with distilled water and centrifuged in 10,000 rpm. Pale white powder of zinc oxide nanoparticle was obtained after drying at 500°C.



Chemical method: 0.10M zinc acetate dihydrate including poly vinyl pyrrolidone (PVP) as structure director was sonicated. 0.28M tetramethylammoniumhydroxide (TMAH) was slowly added. The precipitate was filtered and washed with distilled water and ethanol for three times. Ethanol was added to the obtained precipitate and again sonicated, the sonicated mixture was filtered. The final obtained precipitate was dehydrated at 500°C.

The bio synthetic method employing plant extract have attention as a simple and viable alternative to chemical methods. The biological approach appears to be a cost effective alternative to conventional chemical methods of synthesis. Result proves that the green synthesised nanoparticle shows more enhanced biocidal activity against various pathogens when compared to chemical zinc oxide nanoparticle.

#### CHARACTERIZATION OF ZnO NANOPARTICLES:

##### *X- Ray Diffraction*

The crystalline phase formation and size of ZnO nanoparticles were analysed by X-ray Diffraction (XRD) measurements which was carried out using with Cu(K $\alpha$ ) radiation ( $\lambda = 1.54060 \text{ \AA}$ ) operating at 40 kv and 30 mA with ranging 20-70°. The average particle size of ZnO nanoparticle was determined from XRD patterns of ZnO samples according to Scherrer's equation.  $D = k\lambda / \beta \cos\theta$ , Where k is a constant,  $\lambda$  is the X- ray wavelength which equals to 1.54060nm,  $\beta$  is the full width half maximum intensity (FWHM) and  $\theta$  is the half diffraction angle.

##### *Scanning Electron Microscope*

The external morphology of the sample were characterized by Scanning Electron Microscope (SEM).

##### *Antibacterial activity*

2.8g of nutrient agar was weighed correctly and dissolved in 100ml of sterile distilled water. P<sup>H</sup> was adjusted to 7.2 and was autoclaved at 121°C for 15 minutes. 20 ml of molten agar was poured in to the sterile petriplate and allowed to solidify.

##### *Disc diffusion Assay*

Antibacterial activity of chemical and green synthesised ZnO nanoparticles were seen against gram negative and gram positive bacteria by disc diffusion method. The disc of 4mm prepared using a Whatman No:1 filter paper. The discs were prepared by punching and putting in vials and sterilizing at 150°C for 15 min. The discs were impregnated with 10 ul of concentrated crude extract and another discs were impregnated with 10ul of green and chemical synthesised zinc oxide nanoparticles. The discs were evaporated at 37°C for 24 h. Prepared discs containing the various fractions were carefully placed on the inoculated plates using a sterilized forceps in each case.

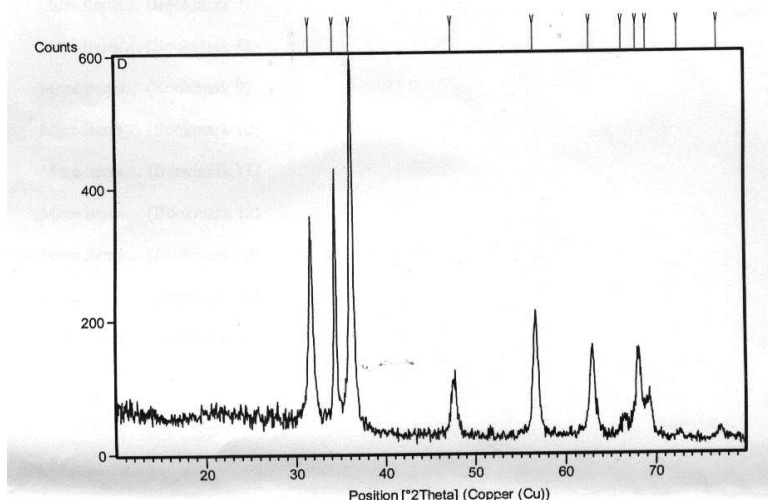
The antibiotic gentamycine were used as control. The plates were then turned upside down and incubated at 37°C for 24 h in an incubator. The results were taken by considering the zone of growth and inhibition of the bacteria by the test fractions. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disc.

#### RESULT AND DISCUSSION

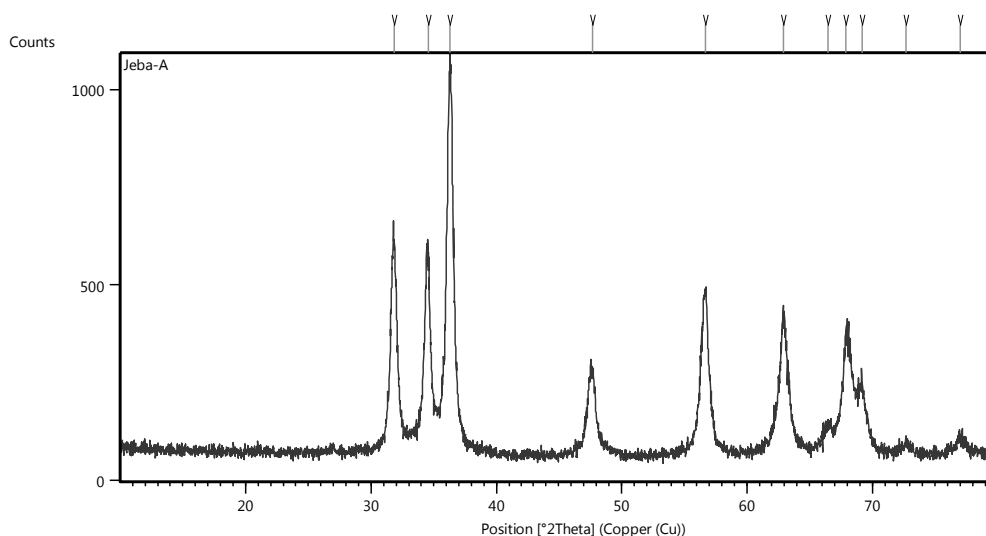
##### *X-ray powder diffraction*

Fig(1) shows the X-ray diffraction patterns of ZnO nanoparticle synthesised by the reaction of Zinc acetate dihydrate with (PVP) and (TMAH). From fig (1) the peaks obtained for chemical method to (101),(002),(100),(110),(103),(112),(102),(202) planes in hexagonal type of ZnO nanoparticle are in high quality record with the values of standard card (2003 JCPDS NO- international center for Diffraction data). More over the broad peaks at about 36° as indicates of nanocrystalline nature of the ZnO phase. No other contamination peaks are observed. From the XRD peak the average grains size was estimated using Scherrer's equation as 3.6nm.

Fig(2) shows X-ray powder diffraction patterns of ZnO nanoparticle synthesized by the reaction of ethanolic extract of Zingiber officinale rhizome with zinc acetate dihydrate and sodium hydroxide. The peaks obtained from this green method is (101),(100),(002),(110),(103),(112),(102),(201),(200),(202) planes in hexagonal type structure. The average particle size was about 4 nm which was probable by Debye Scherrer's equation ( $D = k\lambda / \beta \cos\theta$ )



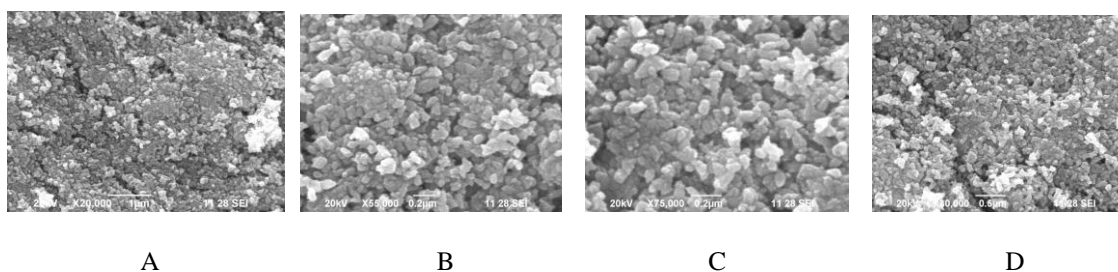
**Figure 1: XRD Spectra of Chemical Synthesis ZnO NP**



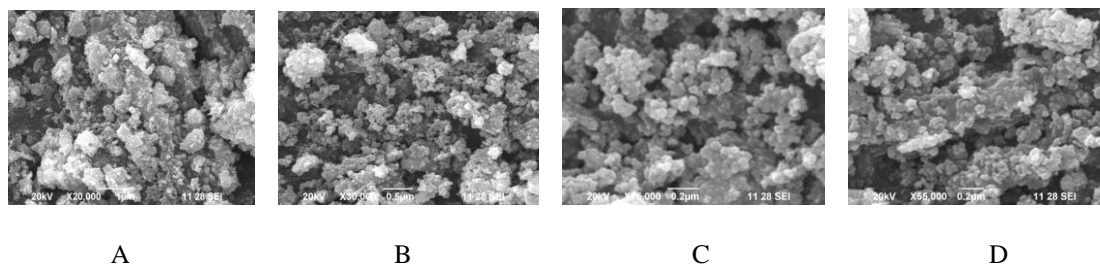
**Figure 2 : XRD Spectra Green Synthesis ZnO NP**

### Scanning Electron Microscope

The SEM analysis has been used to examine the surface morphology of the ZnO nanopowders synthesised by green and chemical method. The figure 3 (A,B,C,D) and 4 (A,B,C,D) shows the surface morphologies of the ZnO nano powders of different magnification for chemical and green method respectively. Both images showed that the spherical shaped individual particles as well as number of aggregates. Since the agglomeration taken place in the prepared samples, it is difficult to find the exact size of the particles. However, the average size of the selected individual particles are in the order of 150 nm for chemical method and 90 nm for green method.



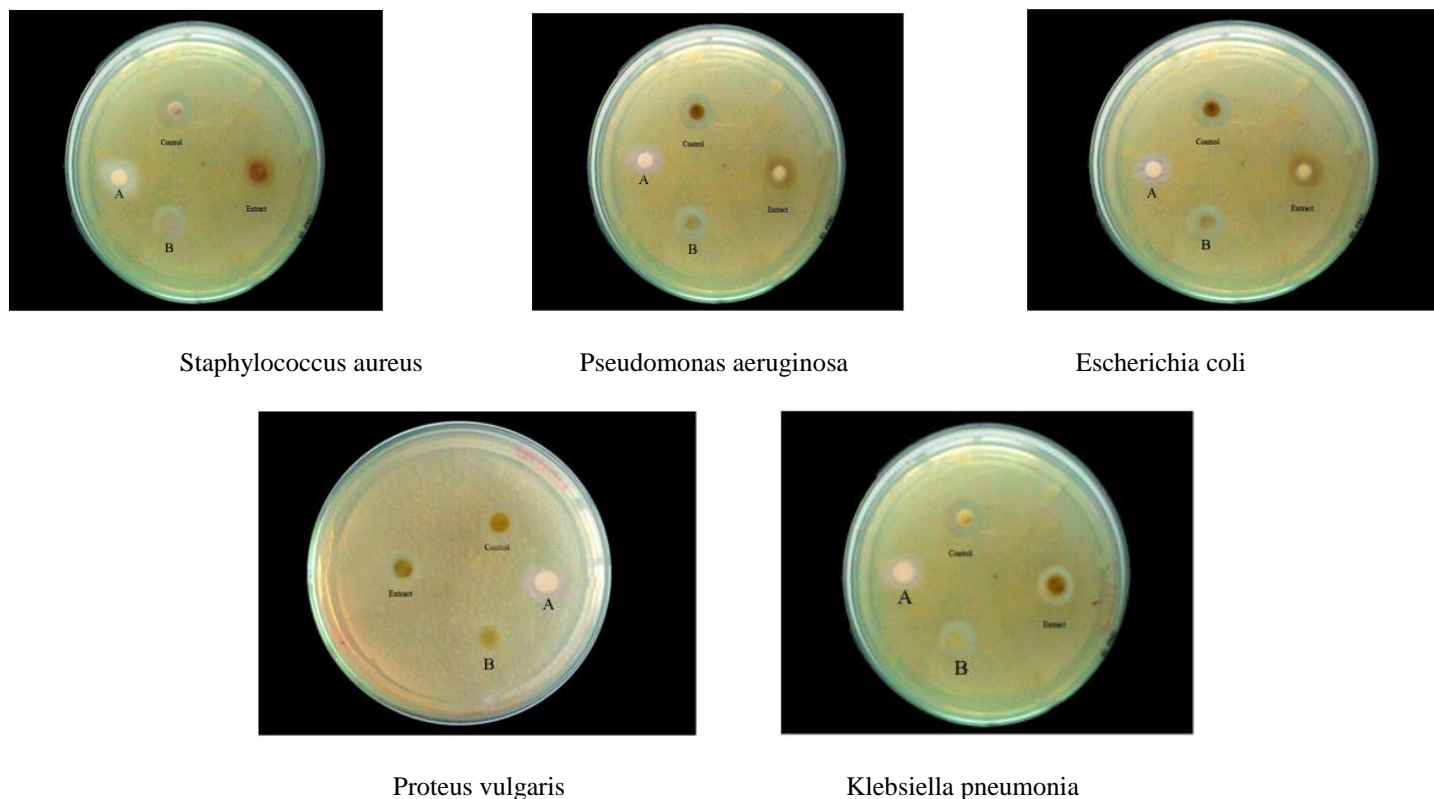
**Figure 3: (A, B, C, D) SEM pictures of Chemical Synthesis ZnO NP**



**Figure 4: (A, B, C, D) SEM pictures of Green Synthesis ZnO NP**

### ***Antibacterial activity***

The antibacterial activity of green and chemical synthesized ZnO suspension towards various bacterias were tested by disc diffusion method. The presence of inhibition zone clearly indicates that the mechanism of the biocidal action of ZnO nanoparticles which involves disruption of the membrane with high rate of generation of surface oxygen species and finally lead to the death of pathogen. The results indicated that ZnO nanoparticle synthesized from chemical and Zingiber officinale extract showed the antibacterial activity both the Gram negative and gram positive bacteria. In our study green synthesized ZnO nanoparticle showed great significant inhibition when compared to chemical synthesized ZnO nanoparticles.



**Figure 5: Antibacterial study of zinc oxide nanoparticles synthesized from Zingiber Officinale against selective bacterial pathogens**

**Table 1: Antibacterial activity of nanoparticles of chemical and green method**

S. No.	Organism	Plant extract(Zingiber Officinale)(mm)	Control-Gentamycin	Sample A-Nanoparticles of chemicalmethod (mm)	Sample B-Nanoparticles of Zingiber Officinale (mm)
1	Staphylococcus aureus	12mm	15mm	9mm	13mm
2	Pseudomonas aeruginosa	10mm	10mm	8mm	12mm
3	Klebsiella pneumoniae	11mm	10mm	10mm	12mm
4	Escherichia coli	10mm	9mm	07mm	14mm
5	Proteus vulgaris	10mm	8mm	07mm	11mm

## SUMMARY

XRD study reveals the average size was 4 nm in green method and 3.6 nm in chemical method. SEM study shows the average size of the selected individual particle is 150 nm for chemical and 90 nm for green method. In this study, we have demonstrated the enhanced bioactivity of green synthesized ZnO nanoparticles by studying the antibacterial activity of suspensions with various other formulations using a standard bacterial method. The growth inhibition was higher in green synthesized ZnO than chemical ZnO nanoparticle and other common antibacterials. From this study, it can be concluded that the ZnO nanoparticles constitute an effective antibacterial against pathogenic microorganisms.

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